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Lymphocyte to monocyte ratio (LMR) predicts mortality in patients with liver cirrhosis

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Abstract

Objective: Infection with hepatitis B virus (HBV) remains a major cause of liver cirrhosis (LC) in China. Recent reports suggest that the lymphocyte to monocyte ratio (LMR) is a potential biomarker for predicting clinical outcomes. In our study, we investigated if LMR can be used as a prognostic marker of mortality in LC patients.

Design: A cross-sectional study.

Setting: HBV-infected patients with LC and patients with chronic hepatitis B infection (CHB) from Department of Infectious Disease were enrolled in our retrospective cohort and 240 healthy individuals were recruited in healthcare centre in the First Affiliated Hospital of Zhejiang University.

Participants: 479 HBV-infected patients with LC, 134 patients with CHB, and 240 healthy individuals were enrolled.

Primary and secondary outcome measures: The receiver operating characteristic (ROC) curve and multivariable logistic regression analysis after adjusting gender, total protein, albumin, total bilirubin and the model for end-stage liver disease (MELD) score, were used to evaluate the power of LMR for predicting mortality following one year in LC patients.

Results: In LC patients, the LMR was statistically lower. The MELD score and mortality were statistically higher than those with chronic hepatitis B (CHB) and control groups. LMR in LC correlated with MELD score (r = 0.323). The area under the ROC curve (AUROC) of LMR for predicting mortality LC was 0.789 (95% confidence interval (CI): 0.735-0.842; $P < 0.001$); the AUROC of 1/LMR+MELD score was 0.885 (95% CI: 0.842-0.928; $P < 0.001$), and the multivariate logistic regression analysis showed that LMR was an independent predictive factor of mortality in LC (odds ratios [OR]: 2.347, 95% CI: [1.134-4.859]; $P = 0.022$).

Conclusion: Our results strongly suggest that low LMR can be considered as an independent biomarker for predicting mortality in patients with LC.

Keywords: liver cirrhosis; lymphocyte to monocyte ratio; the model for end-stage liver disease score

Strengths and limitations of this study

- LMR was lower in LC group, especially in the non-surviving group, compared to the control group and the CHB group.
- LMR was closely correlated to the MELD score.
- When LMR and MELD score were combined, the power for predicting mortality of LC patients were increased.
- Low LMR levels were independent factors for predicting mortality in LC patients.
- This was a retrospective study and validation cohort was lack.

Introduction

Liver cirrhosis (LC) is a common hepatic disease in China, and represents an increasing cause of morbidity and mortality [1,2]. Hepatitis B virus (HBV) infection remains a major cause of LC in China, with a 3% yearly incidence of decompensated cirrhosis [3]. Systemic inflammatory response syndrome (SIRS) is relatively common in patients with complicated cirrhosis [4,5]. SIRS can further deteriorate liver function, maximize the risk of complications and increase the mortality rate of LC patients [4,5]. SIRS is usually measured by peripheral blood count-based parameters, such as neutrophils, lymphocytes, monocytes, red blood cell distribution width (RDW), mean platelet volume (MPV) or platelet count. These parameters have been reported to be independent predictive markers of clinical outcome in cancer and different states of HBV-related hepatic disorders [6-10]. Among these inflammatory parameters, the neutrophil-lymphocyte ratio (NLR), RDW and monocyte ratio have been proposed as easily accessible and reliable markers [6-8,11]. Several recent studies suggest that the lymphocyte to monocyte ratio (LMR) is a cheap, readily available and reproducible test with a potential for predicting clinical outcomes of patients with solid tumors and hematologic malignancy, including nasopharyngeal carcinoma, colorectal cancer, pancreatic cancer, and lymphoma [12-15]. Moreover, Merekoulias *et al.,* found that, in 90% of patients who had influenza virus, lymphopenia and/or monocytosis, LMR could be used as a time-saving and cost-effective screening test for influenza virus infection, leading to early antiviral treatment and a decreased incidence of complications [16]. Assuming that there may be association between LMR and LC severity, we investigated the potential prognostic value of LMR as a biomarker in HBV-related LC.

To the best of our knowledge, there is no data on LMR as a LC diagnostic measure for LC. We therefore investigated, in a retrospective cohort, the association between LMR in peripheral blood in LC patients, with special emphasis on the value of LMR for predicting the mortality of LC patients.

Subjects and Methods

Subjects

There were 134 patients with chronic hepatitis B infection (CHB) and 479 patients with HBV-related liver cirrhosis (LC) from the Department of Infectious Disease, The First Affiliated Hospital, School of Medicine, Zhejiang University, between October 2012 and October 2013, included in our retrospective cohort study. CHB and LC were diagnosed according to the criteria of the 2000 Xi'an viral hepatitis management scheme [17]. The LC group was subdivided into two subgroups according to mortality at one year of follow up. Ninety-two LC patients died of upper gastrointestinal bleeding, hepatic encephalopathy, hepatorenal syndrome, infection, gastrointestinal bleeding and/or hepatic encephalopathy [18-19]. Two hundred and forty healthy controls corresponded to HBsAg negative individuals with normal liver function, normal renal function and no infection. Patients with a concurrent infection of hepatitis C/D/G virus, human immunodeficiency virus, hepatocellular carcinoma, alcoholic cirrhosis,

schistosomiasis cirrhosis and any autoimmune liver disease were excluded.

Ethics statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of the Medical College at Zhejiang University in China and was performed in accordance with the Helsinki Declaration.

Laboratory assessment

All venous blood samples were obtained in the morning following a 12 h fast, within 24 h after admission. All study participants were subjected to the following determinations: serum total protein (TP), albumin (ALB), total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (Tch), creatinine (Cr), prothrombin time (PT), complete blood cell counts, LMR in peripheral blood), international normalized ratio (INR) and the model for end-stage liver disease (MELD) score based on TB, Cr, INR and PT [18]. Complete blood cell counts were determined using a Sysmex XE-2100 automated hematology analyzer (Sysmex Corp, Kobe, Japan) with Sysmex reagents.

Statistical analysis

Statistical analysis was performed using SPSS16.0 (SPSS Inc. IL, USA). Data were presented as mean ± SD, median (range) or categorical data as percentages, if appropriate. The differences between two groups were assessed with an independent sample t-test, the Mann-Whitney U test or chi-square test, if appropriate. Multiple comparisons were performed by one-way analysis of variance (ANOVA) or Kruskal-Wallis H tests, if appropriate. Spearman correlation test was used in correlation analyses. The receiver operating characteristic (ROC) curve and cutoff values of LMR were obtained, and area under ROC curve (AUROC) was calculated to identify the best LMR and/or MELD score for predicting mortality in LC patients. These parameters were selected by stepwise regression, and multivariate logistic regression analyses were used to evaluate if low LMR was an independent factor for predicting mortality in LC patients by unadjusted model and adjusting for gender, TP, ALB, TB and MELD score. The high LMR group was used as the reference category. Statistical significance was defined at $P < 0.05$.

Results

Patients characteristics

There were 479 LC patients, 134 CHB patients, and 240 healthy controls enrolled in our retrospective cohort. The patient characteristics are listed in Table 1. No statistical differences were observed for gender and age between the three groups. Whereas, TP, ALB, TB, ALT, AST, TG, Tch, Cr, INR, LMR, and WBC count were statistically different (all $P < 0.05$). The MELD score and mortality of LC group were statistically higher than those of the CHB group ($P < 0.001$).

LMR is lower in LC, especially in the non-surviving group

The LMR was significantly lower in the LC group as compared to the control group (2.77 *vs.* 5.30, respectively) and to the CHB group (2.77 *vs.* 3.64; $P < 0.01$). The non-surviving group exhibited a lower LMR (1.14) and higher MELD score (17.27) than the surviving group (3.11 and 7.99, respectively; $P < 0.001$) (Fig. 1).

LMR is closely correlated to the MELD score

The LMR in LC group correlated with INR, ALB, Cr, TB, Tch, TG, TP, WBC and MELD score (r = -0.130, 0.127, -0.163, -0.211, 0.233, 0.173, 0.219, -0.288, and -0.241; all $P < 0.05$). In non-surviving LC patients, LMR negatively correlated with MELD score with a higher correlation coefficient (r = -0.354; $P = 0.13$) compared with other indexes. In contrast, in the CHB group, LMR correlations with INR, ALB, TB, WBC, and MELD score were lower (r= -0.266, 0.249, -0.324, -0.186 and -0.266, respectively; all $P < 0.05$), and this was even more pronounced for the control group where correlations with ALB and TP were only 0.198 and 0.142, respectively ($P < 0.05$).

Combining LMR and MELD score increases the power for predicting mortality

The ROC curve analyses were applied to estimate LMR and MELD score to predict mortality of LC patients (Fig. 2). LMR was changed into 1/LMR by inverse transformation. The AUROCs of 1/LMR and MELD score were 0.789 (95% confidence interval (CI): 0.735-0.842; $P < 0.001$) and 0.878 (95% CI: 0.831-0.924; $P < 0.001$), respectively. When 1/LMR and MELD score were combined, the AUC was 0.885 (95% CI: 0.842-0.928; $P < 0.001$). The cutoff values, sensitivity and specificity of MELD were 16.89%, 72.8% and 91.5%. For LMR the values were 2.10%, 77.2%, and 71.8%. When LMR was combined with MELD, the specificity reached up to 97.4%.

The non-surviving patients had a higher level of WBC (6.75 [0.8-24.9] *vs.* 3.6 [0.9-32.8]x10^9/L; $P < 0.001$) and monocytes (0.73 [0.04-3.16] *vs.* 0.33 [0.05-2.0]x10^9/L; $P < 0.001$) than the surviving patients. Although the median and range of lymphocyte count of the non-surviving group were slightly lower than those of the surviving group (0.9 [0.1-4.3] *vs.* 1.00 [0.10-5.40]x10^9/L), the difference did not reach statistical significance ($P = 0.166$). These data indicated that the lower LMR in the death group was mainly due to an increased number of monocytes and secondarily due to decreased lymphocytes.

To further explore the association of LMR and mortality, the 479 LC patients were divided into two groups according to the cutoff value (low LMR: LMR ≤ 2.1 and high LMR: LMR > 2.1). The clinical characteristics and differences in variables between these groups are presented in Table 2. Patients with low LMR values had higher mortality, MELD score, TB, ALT, AST, Cr, INR and WBC, and had lower TP, ALB and Tch, compared with the high LMR subgroup.

LMR is an independent prognostic factor of mortality in multivariate analysis

Gender, MELD, low LMR (with high LMR as reference), TP, TB and ALB were

selected by stepwise regression from the above parameters (P = 0.025, < 0.001, 0.048, 0.006, < 0.001 and 0.021, respectively) with forward selection. Subsequent multivariate logistic regression analysis showed that low LMR levels were independent factors for predicting mortality in LC patients (Table 3).

Discussion

In the present retrospective study of HBV-LC a significant negative association was found between LMR in the peripheral blood and the MELD score. LMR of LC patients was statistically lower, and the MELD score and mortality of LC patients were statistically higher than those of CHB and control groups, especially in the non-surviving LC subgroup. Moreover, low LMR was an independent predictive factor of mortality. These results provide the first evidence for an association between LMR and mortality in LC patients.

Bacterial infections are an important cause of morbidity and mortality in patients with LC due to an impaired immune function together with an increased passage of bacteria from the gut (bacterial translocation [BT]) [4,5,20]. Once infection occurs, it may lead to SIRS, which can cause serious complications such as severe sepsis, renal dysfunction, encephalopathy, coagulopathy and multiple organ failure [20]. SIRS occurs more frequently in patients with advanced cirrhosis and portal hypertension, and is associated with severity of liver disease and increased risk of death in LC patients [4,5]. The mortality of LC patients with infection has been reported to be more than twice that of patients without infection [20]. Monocytes are central mediators of the immune response and play a crucial role in the pathogenesis of liver cirrhosis. Endotoxin leads to monocyte activation and promotes the release into the serum of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNFα), and interferon-γ. This release is proportional to liver disease severity. These cytokines act in an autocrine and paracrine fashion and result in the recruitment of inflammatory effector cells, such as polymorphonuclear cells [20-22]. The subsequent activation of nitric oxide (NO) via the cytokine cascade leads to vasodilatation [24]. Endotoxin, cytokines and NO are key elements in the pathogenesis of circulatory abnormalities in liver cirrhosis with infection. Li *et al.,* found that monocytes in HBV-related LC patients positively correlated with the endotoxin level and cirrhosis severity based on the Child-pugh classification, indicating that the endotoxin-driven monocyte activation was an important factor of SIRS and multiple organ failure [25]. Lee *et al.*, found that LC patients with hepatocellular carcinoma had a high monocyte ratio and that a preoperative monocyte ratio > 7% was an independent risk factor for survival after hepatic resection [11]. Immune paralysis, defined as decreased human leukocyte antigen-DR (HLA-DR) expression on monocytes and indicating immune dysfunction, was found in LC patients. HLA-DR expression is a direct marker of monocyte function and a protective immune response in LC patients [23]. Monocyte HLA-DR expression is significantly reduced in those patients and falls in proportion to cirrhosis severity [26,27]. Therefore, LC patients may have high monocyte count but low monocyte HLA-DR expression for systemic inflammatory response and immune paralysis. Early diagnosis and treatment of infections can significantly reduce

morbidity and improve survival of LC patients [4,5,21,23,24,28].

Inflammatory stimuli mainly affect the numbers of monocytes in the peripheral blood in LC patients, which contributes to LMR changes. In addition, the present study showed that lymphocytes in the death group showed a trend towards lower levels as compared with the survival group, without reaching statistical significance. Such a decline might be attributed to lymphocytopenia [29,30]. This is in accordance with Leithead *et al.,* who found that a lower lymphocyte count was associated with mortality in patients with end-stage cirrhosis listed for liver transplantation [29]. Lombardo *et al.,* also found that the progressive and severity-related decrease in peripheral blood T-lymphocyte suggested a progressive impairment of protective immune function in LC [30]. Therefore, high monocytes together with low lymphocytes may reflect the severity and progression of liver injury in LC patients.

LMR has been shown to be associated with tuberculosis and influenza virus infection [16,31]. Recently, LMR has also been reported to predict survival and prognosis in various patient populations with malignant diseases [12-15], and a decreased LMR has been shown to be significantly associated with a high risk for critical limb ischemia in peripheral arterial occlusive disease patients [32]. Compared with another novel inflammation index, the ability of NLR for predicting mortality (AUROC) in LC patients [33] was similar to LMR in our study. LMR was associated, in our study, with MELD score and was an independent predictive factor of mortality. Combined with the MELD score, the specificity for predicting mortality was improved. Additionally, the LMR is an easily available and low price biomarker. However, it should be noted that this was a retrospective study so that validation cohorts are warranted in order to confirm the present data. Moreover, these findings may only apply to HBV-related LC patients and, therefore, need to be validated in other etiologies of liver cirrhosis by future prospective clinical trials.

Contributors L.F. designed the experiments. G. F., JW.Z. J.Z performed the experiments. Y.Z. and J.Y. analysed and interpreted all the data. J.Z and Y.Z. wrote the main manuscript text. All authors reviewed the manuscript.

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Competing interests None.

Patient consent Obtained.

Ethics approval This study was approved by the ethics committee of the First Affiliated Hospital of Zhejiang University School of Medicine, China.

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Figure Legends

Figure 1: The boxplots of MELD score and LMR between surviving and non-surviving LC patients
LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score.

Figure 2: Receiver operating characteristic (ROC) curve analysis for predicting mortality by LMR and MELD score
LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score; 1/LMR+MELD, 1/LMR combined with MELD.

Table 1. Basic characteristics of enrolled participants.

Variables	Control (240)	CHB (134)	LC (479)	*P* value
Female/male	61/179	34/100	126/353	0.956
Age (year)	50.6±9.69	48.9±8.04	50.8±10.8	0.163
HBsAg positive (yes/no)	0/240	134/0	479/0	-
HBeAg positive (yes/no)	0/240	66/68	184/295	0.024#
TP (g/L)	71.6±3.79	67.3±6.83*	62.9±8.48*#	<0.001
ALB (g/L)	46.2±3.17	37.4±5.95*	33.2±5.61*#	<0.001
TBIL (μmol/L)	12(6-49)	21.5(5-309)*	31(5-839)*#	<0.001
ALT (U/L)	17(7-48)	61(9-1838)*	29(4-1882)*#	<0.001
AST (U/L)	19(12-46)	48(16-1235)*	40(8-4094)*#	<0.001
TG (mmol/L)	1.08(0.41-1.70)	1.33(0.44-4.14)*	0.79(0.3-3.59)*#	<0.001
Tch (mmol/L)	4.66(2.40-5.86)	4.04(1.6-8.17)*	2.89(0.74-9.73)*	<0.001
Cr (μmol/L)	73(39-100)	65(29-154)*	66(30-729)*	0.002
INR	0.94±0.05	1.21±0.23*	1.55±0.78*#	<0.001
WBC (10^{12}/L)	5.6(4.0-9.4)	4.75(2-12)*	3.9(0.8-32.8)*#	<0.001
LMR	5.30(1.4-13.2)	3.64(0.65-9.61)*	2.77(0.27-18.25)*#	<0.001
MELD score	-	5.89(0-23.63)	9.89(0.0-57.17)	<0.001 #
Mortality (yes/no)	-	1/133	92/387	<0.001 #

Data were presented as mean ± SD and median (range). CHB, chronic hepatitis B; LC, liver cirrhosis; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score. *P*-value: Comparison among these three groups. #: LC group *vs.* CHB group. *: $P < 0.05$ *vs.* the Control group

Table 2. Clinical characteristics of LC patients according to LMR cutoff value.

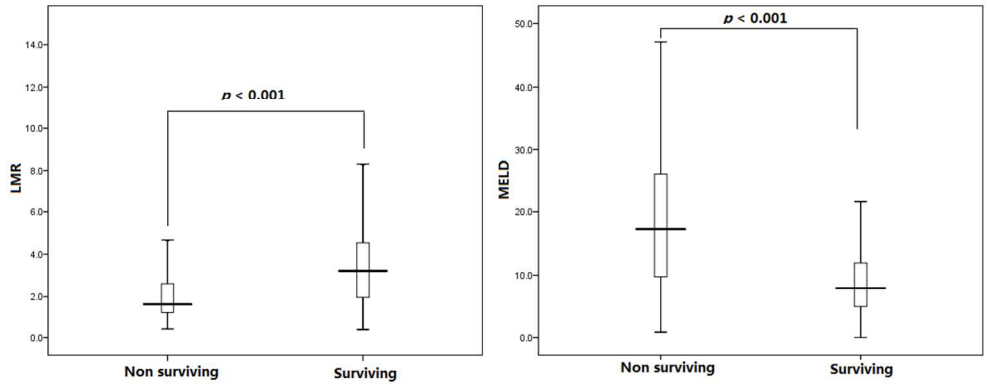
Variables	Low LMR (LMR ≤ 2.10, n=180)	High LMR (LMR > 2.10, n=299)	*P* value
Female/male	52/128	74/225	0.319
Age (year)	51.5±10.1	50.4±11.2	0.290
TP (g/L)	60.6±8.97	64.4±7.87	<0.001
ALB (g/L)	32.3±5.88	33.7±5.40	0.009
TBIL (μmol/L)	50.5(8-839)	26(5-567)	<0.001
ALT (U/L)	31(5-1882)	28(4-1141)	0.468
AST (U/L)	44(16-4094)	37(8-1078)	<0.001
TG (mmol/L)	0.78(0.32-2.15)	0.79(0.30-3.59)	0.229
Tch (mmol/L)	2.37(0.79-5.29)	3.07(0.74-9.73)	<0.001
Cr (μmol/L)	72(30-729)	65(30-426)	<0.001
INR	1.82±1.17	1.39±0.29	<0.001
WBC (10^{12}/L)	5.20(0.8-32.8)	3.5(0.8-12.1)	<0.001
MELD score	14.52(0-57.2)	8.20(0.0-41.25)	<0.001
Mortality (yes/no)	71/109	21/278	<0.001

Data were presented as mean ± SD and median (range). LMR, lymphocyte to monocyte ratio; LC, liver cirrhosis; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; MELD score, model for end-stage liver disease score.

Table 3. Odds ratios of low LMR for predicting mortality in LC patients

Models	Odds Ratio (95% CI)	*P* value
Model 1	8.623 (5.051-14.721)	< 0.001
Model 2	8.565 (5.013-14.634)	< 0.001
Model 3	3.392 (1.724-6.670)	< 0.001
Model 4	2.347 (1.134-4.859)	0.022

Odds ratios of low LMR were determined using high LMR as reference; model 1: unadjusted; model 2: adjusted for gender; model 3: adjusted for gender, TP, ALB, and TB; model 4: adjusted for gender, TP, ALB, TB and MELD score.



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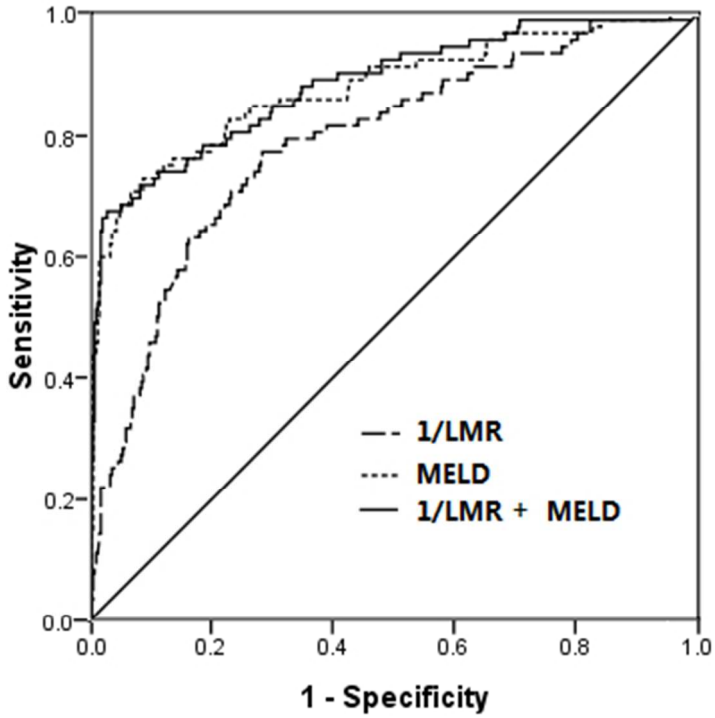

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STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	no
Study size	10	Explain how the study size was arrived at	no
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5
		(*b*) Describe any methods used to examine subgroups and interactions	5
		(*c*) Explain how missing data were addressed	no
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	5

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	5
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5,table 1
		(b) Give reasons for non-participation at each stage	no
		(c) Consider use of a flow diagram	no
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5,table 1
		(b) Indicate number of participants with missing data for each variable of interest	no
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	no
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	5-6,table 1-2
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6,table 3
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-7,table 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
Discussion			
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-8
Generalisability	21	Discuss the generalisability (external validity) of the study results	7-8
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	8

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between lymphocyte to monocyte ratio (LMR) and the mortality of liver cirrhosis: A retrospective cohort study

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Association between lymphocyte to monocyte ratio (LMR) and the mortality of liver cirrhosis: A retrospective cohort study

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Abstract

Objective: Infection with hepatitis B virus (HBV) remains a major cause of liver cirrhosis (LC) in China. Recent reports suggest that the lymphocyte to monocyte ratio (LMR) is a potential biomarker for predicting clinical outcomes. In our study, we investigated if LMR can be used as a prognostic marker of mortality in LC patients.

Design: A retrospective cohort study.

Setting: HBV-infected patients with LC and patients with chronic hepatitis B infection (CHB) from the Department of Infectious Disease were enrolled and 240 healthy individuals were recruited from the healthcare center at the First Affiliated Hospital of Zhejiang University.

Participants: 479 HBV-infected patients with LC, 134 patients with CHB, and 240 healthy individuals were enrolled.

Primary and secondary outcome measures: The receiver operating characteristic (ROC) curve and multivariable logistic regression analysis after adjusting gender, total protein, albumin, total bilirubin and the model for end-stage liver disease (MELD) score, were used to evaluate the power of LMR for predicting 1-year mortality in LC patients.

Results: The LMR was statistically lower in LC patients. The MELD score and mortality were statistically higher in LC patients compared to those with chronic hepatitis B (CHB) and control groups. The area under the ROC curve (AUROC), cutoff values, sensitivity, and specificity of LMR for predicting mortality LC in the training cohort were 0.817 (95% confidence interval (CI): 0.746 - 0.888; $P < 0.001$), 2.10, 82.6, and 78.8%, and these data were confirmed in the validation cohort. The multivariate logistic regression analysis showed that LMR was an independent predictive factor of mortality in LC (odds ratios [OR]: 2.347, 95% CI: [1.134 - 4.859]; $P = 0.022$).

Conclusion: Our results strongly suggest that low LMR can be considered as an independent biomarker for predicting mortality in patients with LC.

Keywords: liver cirrhosis; lymphocyte to monocyte ratio; the model for end-stage liver disease score

Strengths and limitations of this study

- LMR was lower in the LC group, especially in the non-surviving group, compared to the control group and the CHB group.
- LMR was closely correlated to the MELD score.
- LMR was an easy parameter to achieve and the power for predicting mortality of LMR was similar to that of MELD.
- Low LMR levels were independent factors for predicting mortality in LC patients.
- This was a retrospective study.

Introduction

Liver cirrhosis (LC) is a common hepatic disease in China, and represents an increasing cause of morbidity and mortality [1,2]. Hepatitis B virus (HBV) infection remains a major cause of LC in China, with a 3% yearly incidence of decompensated cirrhosis [3]. Systemic inflammatory response syndrome (SIRS) is relatively common in patients with complicated cirrhosis [4,5]. SIRS can further deteriorate liver function, maximize the risk of complications and increase the mortality rate of LC patients [4,5]. SIRS is usually measured by peripheral blood count-based parameters, such as neutrophils, lymphocytes, monocytes, red blood cell distribution width (RDW), mean platelet volume (MPV) or platelet count. These parameters have been reported to be independent predictive markers of clinical outcome in cancer and different states of HBV-related hepatic disorders [6-10]. Among these inflammatory parameters, the neutrophil-lymphocyte ratio (NLR), RDW and monocyte ratio have been proposed as easily accessible and reliable markers [6-8,11]. Several recent studies suggest that the lymphocyte to monocyte ratio (LMR) is a cheap, readily available and reproducible test with potential for predicting clinical outcomes of patients with solid tumors and hematologic malignancy, including nasopharyngeal carcinoma, colorectal cancer, pancreatic cancer, and lymphoma [12-15]. Moreover, Merekoulias *et al.,* found that, in 90% of patients who had influenza virus, lymphopenia and/or monocytosis, LMR could be used as a time-saving and cost-effective screening test for influenza virus infection, leading to early antiviral treatment and a decreased incidence of complications [16]. Assuming that there may be association between LMR and LC severity, we investigated the potential prognostic value of LMR as a biomarker in HBV-related LC.

To the best of our knowledge, there is no data on LMR as a LC diagnostic measure. We therefore performed a retrospective cohort study to investigate, the association between LMR in peripheral blood in LC patients, with special emphasis on the value of LMR for predicting the mortality of LC patients.

Subjects and Methods

Subjects

We continuously analyzed all 547 patients with HBV-related liver cirrhosis (LC) from the Department of Infectious Disease, The First Affiliated Hospital, School of Medicine, Zhejiang University, between October 2012 and October 2013. Sixty-eight LC patients with a concurrent infection of hepatitis C/D/E/G virus (n = 3), human immunodeficiency virus (HIV, n = 1), hepatocellular carcinoma (HCC, n = 56), alcoholic cirrhosis (n = 5), schistosomiasis cirrhosis (n = 1), and any autoimmune liver disease (n = 2) were excluded. The remaining 479 LC patients were enrolled in our retrospective cohort study. All clinical data were retrieved from medical records at the Department of Infectious Disease. One hundred thirty-four patients with chronic hepatitis B infection (CHB), with no statistical differences in age and gender versus LC patients, were selected from the Department of Infectious Disease without a concurrent infection of hepatitis C/D/G virus, HIV, HCC, and any autoimmune liver

disease. CHB and LC were diagnosed according to the criteria of the 2000 Xi'an viral hepatitis management scheme [17]. Liver cirrhosis was diagnosed based on the history of liver disease, clinical manifestations, laboratory tests, imaging tests, and, whenever feasible, liver biopsy[17]. CHB was defined as previous hepatitis B or hepatitis B surface antigen (HBsAg) positivity for > 6 months and persistently positive HBsAg and/or HBV DNA[17-18]. The LC group was subdivided into two subgroups according to mortality at 1-year of follow up. For LC and CHB patients discharged from hospital, 1-year prognostic information was obtained by checking medical records or by contacting the patients' family members. One hundred and eight LC patients were decompensated. Out of 92 LC patients mainly died of upper gastrointestinal bleeding (n = 40), hepatic encephalopathy (n = 28), hepatorenal syndrome (n = 15), infection (n = 5), or of other causes (n = 4). Two hundred and forty healthy controls with no statistical differences in age and gender versus LC patients were selected from health examination population who underwent a general health checkup that included a physical examination and some clinical laboratory tests at the Health Care Centre of the First Affiliated Hospital of Medical College of Zhejiang University. They corresponded to HBsAg negative individuals with normal liver function, normal renal function, and no infection. One hundred and thirty-four CHB patients and 240 healthy controls were used to compare basic characteristics with 479 LC patients.

Ethics statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of the Medical College at Zhejiang University in China and was performed in accordance with the Helsinki Declaration.

Laboratory assessment

All venous blood samples were obtained in the morning following a 12 h fast, within 24 h after admission. All study participants were subjected to the following determinations: serum total protein (TP), albumin (ALB), total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (Tch), creatinine (Cr), prothrombin time (PT), complete blood cell counts, LMR in peripheral blood), international normalized ratio (INR) and the model for end-stage liver disease (MELD) score based on TB, Cr, INR and PT [18]. Complete blood cell counts were determined using a Sysmex XE-2100 automated hematology analyzer (Sysmex Corp, Kobe, Japan) with Sysmex reagents.

Statistical analysis

Statistical analysis was performed using SPSS16.0 (SPSS Inc. IL, USA). Data were presented as mean ± SD, median (range) or categorical data as percentages, if appropriate. The differences between two groups were assessed with an independent sample t-test, the Mann-Whitney U test or chi-square test, if appropriate. Multiple comparisons were performed by one-way analysis of variance (ANOVA) or Kruskal-Wallis H tests, if appropriate. Spearman correlation test was used in correlation analyses. The receiver operating characteristic (ROC) curve and cutoff

values of LMR were obtained, and area under ROC curve (AUROC) was calculated to identify the best LMR and/or MELD score for predicting mortality in LC patients. For AUROC analysis of combined 1/LMR and MELD score for predicting mortality in LC patients, predictive models of 1/LMR, MELD, and 1/LMR + MELD were first developed by binary logistic regression analyses, respectively. Probabilities of 1/LMR, MELD, and 1/LMR + MELD were then generated, respectively, and used as three new input variables for the ROC curve analysis (shown in Figure 2). These parameters were selected by stepwise regression, and multivariate logistic regression analyses were used to evaluate if low LMR was an independent factor for predicting mortality in LC patients by an unadjusted model and adjusting for gender, TP, ALB, TB and MELD score. The high LMR group was used as the reference category. Statistical significance was defined at $P < 0.05$.

Results

Patient characteristics

There were 479 LC patients, 134 CHB patients, and 240 healthy controls enrolled in our study. The patient characteristics are listed in Table 1. No statistical differences were observed for gender and age between the three groups. Whereas, TP, ALB, TB, ALT, AST, TG, Tch, Cr, INR, LMR, and WBC count had statistically differences (all $P < 0.05$). The MELD score and mortality of the LC group were statistically higher than those of the CHB group ($P < 0.001$).

LMR is lower in LC, especially in the non-surviving group

The LMR was significantly lower in the LC group compared to the control group (2.77 *vs.* 5.30, respectively) and to the CHB group (2.77 *vs.* 3.64; $P < 0.01$). The clinical characteristics and differences in variables between non-surviving and surviving LC patients are presented in Table 2. The non-surviving patients had lower LMR (Fig. 1), TP, ALB, and Tch, and higher MELD score, TB, ALT, AST, TG, Cr, INR, WBC, monocytes, and rate of decompensated cirrhosis, compared with surviving patients. The median and range of lymphocyte count of the non-surviving group were slightly lower than those of the surviving group, but the difference did not reach statistical significance. These data indicate that the lower LMR in the non-surviving group was mainly due to an increased number of monocytes and secondarily due to decreased lymphocytes. LMR resulted in no significant differences in LC patients whose primary cause of death was upper gastrointestinal bleeding, hepatic encephalopathy, or hepatorenal syndrome ((1.35[0.35-17.75]), 1.42[0.27-18.20], 1.39[0.39-18.25], p=0.955).

LMR is closely correlated to the MELD score

The LMR in the LC group negatively correlated with MELD score (r = -0.241; $P < 0.05$), especially in non-surviving LC patients, LMR negatively correlated with MELD score with a higher correlation coefficient (r = -0.354; $P = 0.013$) compared with LMR in surviving LC patients.

The power for predicting 1-year mortality of LMR

The enrolled 479 LC patients were divided into two cohorts: the training cohort (n = 239) and the validation cohort (n = 240). The ROC curve analyses of the training cohort were applied to estimate LMR and MELD score to predict mortality of LC patients (Fig. 2). LMR was changed into 1/LMR by inverse transformation. The AUROCs of 1/LMR and MELD score were 0.817 (95% confidence interval (CI): 0.746 - 0.888; $P < 0.001$) and 0.868 (95% CI: 0.795 - 0.941; $P < 0.001$), respectively. The cutoff values, sensitivity and specificity of MELD were 19.1, 73.9 and 96.4%. LMR values were 2.10, 82.6, and 78.8%. When 1/LMR and MELD score were combined, the AUC was 0.876 (95% CI: 0.808 - 0.945; $P < 0.001$), only slightly higher than AUC of MELD score, and neither the specificity (71.7%) nor the sensitivity (96.9%) was significantly improved. Applying the LMR to the validation cohort, the AUROCs of 1/LMR, MELD score, and 1/LMR+MELD were 0.773 (95% CI: 0. 692 - 0.854; $P < 0.001$), 0.887 (95% CI: 0.829 - 0.945; $P < 0.001$), 0.890 (95% CI: 0.836 - 0.944; $P < 0.001$), respectively. There were no significant differences in the AUCs of LMR between the estimation and validation cohorts ($Z = 0.741$, $P = 0.053$). To summarize, LMR was an easy parameter to achieve and the power for predicting mortality of LMR was similar to that of MELD.

LMR is an independent prognostic factor of mortality in multivariate analysis

Gender, MELD, low LMR (LMR ≤ 2.10, with high LMR > 2.10 as a reference), TP, TB and ALB were selected by stepwise regression from the above parameters ($P = 0.025$, < 0.001, 0.048, 0.006, < 0.001 and 0.021, respectively) with forward selection. Subsequent multivariate logistic regression analysis showed that low LMR was an independent factor for predicting mortality in LC patients (Table 3).

Discussion

In the present retrospective study of HBV-LC a significant negative association was found between LMR in the peripheral blood and the MELD score. LMR of LC patients was statistically lower, and the MELD score and mortality of LC patients were statistically higher than those of CHB and control groups, especially in the non-surviving LC subgroup. Moreover, low LMR was an independent predictive factor of mortality. These results provide the first evidence for an association between LMR and mortality in LC patients.

Bacterial infections are an important cause of morbidity and mortality in patients with LC due to an impaired immune function together with an increased passage of bacteria from the gut (bacterial translocation [BT]) [4,5,19]. Once infection occurs, it may lead to SIRS, which can cause serious complications such as severe sepsis, renal dysfunction, encephalopathy, coagulopathy and multiple organ failure [19]. SIRS occurs more frequently in patients with advanced cirrhosis and portal hypertension, and is associated with severity of liver disease and increased risk of death in LC patients [4,5]. The mortality of LC patients with infection has been reported to be more than twice that of patients without infection [19]. Monocytes are central mediators of the immune

response and play a crucial role in the pathogenesis of liver cirrhosis. Endotoxin leads to monocyte activation and promotes the release of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNFα), and interferon-γ into the serum. This release is proportional to liver disease severity. These cytokines act in an autocrine and paracrine fashion and result in the recruitment of inflammatory effector cells, such as polymorphonuclear cells [19-21]. The subsequent activation of nitric oxide (NO) *via* the cytokine cascade leads to vasodilatation [22]. Endotoxin, cytokines and NO are key elements in the pathogenesis of circulatory abnormalities in liver cirrhosis with infection. Li *et al.,* found that monocytes in HBV-related LC patients positively correlated with the endotoxin level and cirrhosis severity based on the Child-pugh classification, indicating that the endotoxin-driven monocyte activation was an important factor of SIRS and multiple organ failure [23]. Lee *et al.*, found that LC patients with hepatocellular carcinoma had a high monocyte ratio and that a preoperative monocyte ratio > 7% was an independent risk factor for survival after hepatic resection [11]. Immune paralysis, defined as decreased human leukocyte antigen-DR (HLA-DR) expression on monocytes and indicating immune dysfunction, was found in LC patients. HLA-DR expression is a direct marker of monocyte function and a protective immune response in LC patients [24]. Monocyte HLA-DR expression is significantly reduced in those patients and falls in proportion to cirrhosis severity [25,26]. Therefore, LC patients may have high monocyte count but low monocyte HLA-DR expression for systemic inflammatory response and immune paralysis. Early diagnosis and treatment of infections can significantly reduce morbidity and improve survival of LC patients [4,5,20,22,23,27].

Inflammatory stimuli mainly affect the numbers of monocytes in the peripheral blood in LC patients, which contributes to LMR changes. In addition, the present study showed that lymphocytes in the non-survival group showed a trend towards lower levels as compared with the survival group, without reaching statistical significance. Such a decline might be attributed to lymphocytopenia [28,29]. This is in accordance with Leithead *et al.,* who found that a lower lymphocyte count was associated with mortality in patients with end-stage cirrhosis listed for liver transplantation [26]. Lombardo *et al.,* also found that the progressive and severity-related decrease in peripheral blood T-lymphocyte suggested a progressive impairment of protective immune function in LC [29]. Therefore, high monocytes together with low lymphocytes may reflect the severity and progression of liver injury in LC patients.

LMR has been shown to be associated with tuberculosis and influenza virus infection [16,30]. Recently, LMR has also been reported to predict survival and prognosis in various patient populations with malignant diseases [12-15], and a decreased LMR has been shown to be significantly associated with a high risk for critical limb ischemia in peripheral arterial occlusive disease patients [31]. Compared with another novel inflammation index, the ability of NLR for predicting mortality (AUROC) in LC patients [32] was similar to LMR in our study. LMR was associated, in our study, with MELD score, the power for predicting mortality of LMR was similar to that of MELD, and was an independent predictive factor of mortality. In addition, the LMR is an easily available and low price biomarker. However, it should be noted that this was a

retrospective study so that prospective cohorts are warranted in order to confirm the present data. Moreover, these findings may only apply to HBV-related LC patients and, therefore, need to be validated in other etiologies of liver cirrhosis by future prospective clinical trials.

Contributorship statement L.F. designed the experiments. G. F., JW.Z. J.Z performed the experiments. Y.Z. and J.Y. analyzed and interpreted all the data. J.Z and Y.Z. wrote the main manuscript text. All authors reviewed the manuscript.

Competing interests None declared.

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Ethics approval This study was approved by the ethics committee of the First Affiliated Hospital of Zhejiang University School of Medicine, China. Patient consent Obtained.

Data sharing statement No additional unpublished data are available.

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Figure Legends

Figure 1: The boxplots of MELD score and LMR between surviving and non-surviving LC patients
LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score.

Figure 2: Receiver operating characteristic (ROC) curve analysis for predicting mortality by LMR and MELD score in the training cohort.
LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score; 1/LMR+MELD, 1/LMR combined with MELD.

Table 1. Basic characteristics of enrolled participants.

Variables	Control (240)	CHB (134)	LC (479)	*P* value
Female/male	61/179	34/100	126/353	0.956
Age (year)	50.6±9.69	48.9±8.04	50.8±10.8	0.163
HBsAg positive (yes/no)	0/240	134/0	479/0	-
HBeAg positive (yes/no)	0/240	66/68	184/295	0.024#
TP (g/L)	71.6±3.79	67.3±6.83*	62.9±8.48*#	<0.001
ALB (g/L)	46.2±3.17	37.4±5.95*	33.2±5.61*#	<0.001
TBIL (μmol/L)	12(6-49)	21.5(5-309)*	31(5-839)*#	<0.001
ALT (U/L)	17(7-48)	61(9-1838)*	29(4-1882)*#	<0.001
AST (U/L)	19(12-46)	48(16-1235)*	40(8-4094)*#	<0.001
TG (mmol/L)	1.08(0.41-1.70)	1.33(0.44-4.14)*	0.79(0.3-3.59)*#	<0.001
Tch (mmol/L)	4.66(2.40-5.86)	4.04(1.6-8.17)*	2.89(0.74-9.73)*	<0.001
Cr (μmol/L)	73(39-100)	65(29-154)*	66(30-729)*	0.002
INR	0.94±0.05	1.21±0.23*	1.55±0.78*#	<0.001
WBC (10^{12}/L)	5.6(4.0-9.4)	4.75(2-12)*	3.9(0.8-32.8)*#	<0.001
LMR	5.30(1.4-13.2)	3.64(0.65-9.61)*	2.77(0.27-18.25)*#	<0.001
MELD score	-	5.89(0-23.63)	9.89(0-57.17)	<0.001 #
Mortality (yes/no)	-	1/133	92/387	<0.001 #

Data were presented as mean ± SD and median (range). CHB, chronic hepatitis B; LC, liver cirrhosis; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score. *P*-value: Comparison among these three groups. #: LC group *vs.* CHB group. *: $P < 0.05$ *vs.* the Control group

Table 2. The clinical characteristics and differences in variables between non-surviving and surviving LC patients.

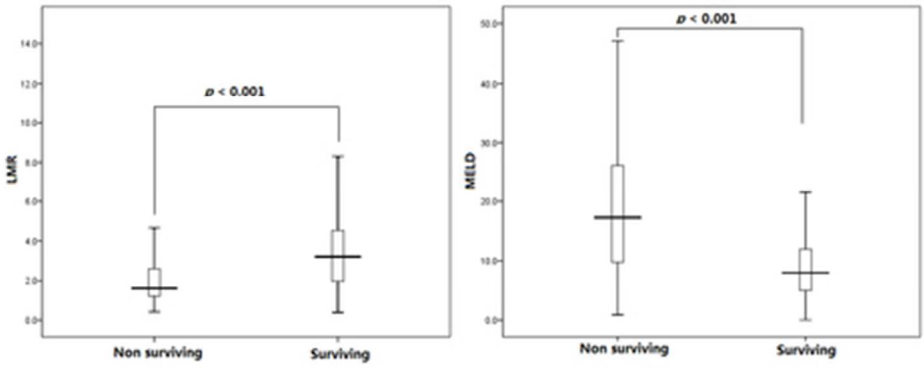
Variables	Non-surviving (n=92)	Surviving (n=387)	*P* value
Female/male	30/62	96/291	0.127
Age (year)	53.8±10.3	50.1±10.8	0.003
TP (g/L)	56.4±8.40	64.5±7.74	<0.001
ALB (g/L)	29.7±5.17	34.0±5.40	<0.001
TBIL (μmol/L)	292.5(9-839)	27(5-836)	<0.001
ALT (U/L)	48(4-1882)	27(5-475)	<0.001
AST (U/L)	66(10-4094)	37(8-440)	<0.001
TG (mmol/L)	0.88(0.30-2.15)	0.76(0.33-3.59)	0.022
Tch (mmol/L)	1.83(0.74-5.29)	3.02(0.94-9.73)	<0.001
Cr (μmol/L)	73.5(30-729)	65(30-326)	<0.001
INR	2.23±1.51	1.39±0.28	<0.001
WBC (10^9/L)	6.75(0.8-24.9)	3.6(0.9-32.8)	<0.001
Monocytes(10^9/L)	0.73 (0.04-3.16)	0.33 (0.05-2.0)	<0.001
Lymphocyte(10^9/L)	0.9 (0.1-4.3)	1.00 (0.10-5.40)	0.166
LMR	1.41(0.27-18.25)	3.10(0.38-14.58)	<0.001
MELD score	22.94(0.84-57.17)	8.49(0-35.33)	<0.001
Decompensated cirrhosis (yes/no)	82/10	26/361	<0.001

Data were presented as mean ± SD and median (range). LMR, lymphocyte to monocyte ratio; LC, liver cirrhosis; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; MELD score, model for end-stage liver disease score.

Table 3. Odds ratios of low LMR for predicting mortality in LC patients

Models	Odds Ratio (95% CI)	*P* value
Model 1	8.623 (5.051-14.721)	< 0.001
Model 2	8.565 (5.013-14.634)	< 0.001
Model 3	3.392 (1.724-6.670)	< 0.001
Model 4	2.347 (1.134-4.859)	0.022

Odds ratios of low LMR were determined using high LMR as reference; model 1: unadjusted; model 2: adjusted for gender; model 3: adjusted for gender, TP, ALB, and TB; model 4: adjusted for gender, TP, ALB, TB and MELD score.



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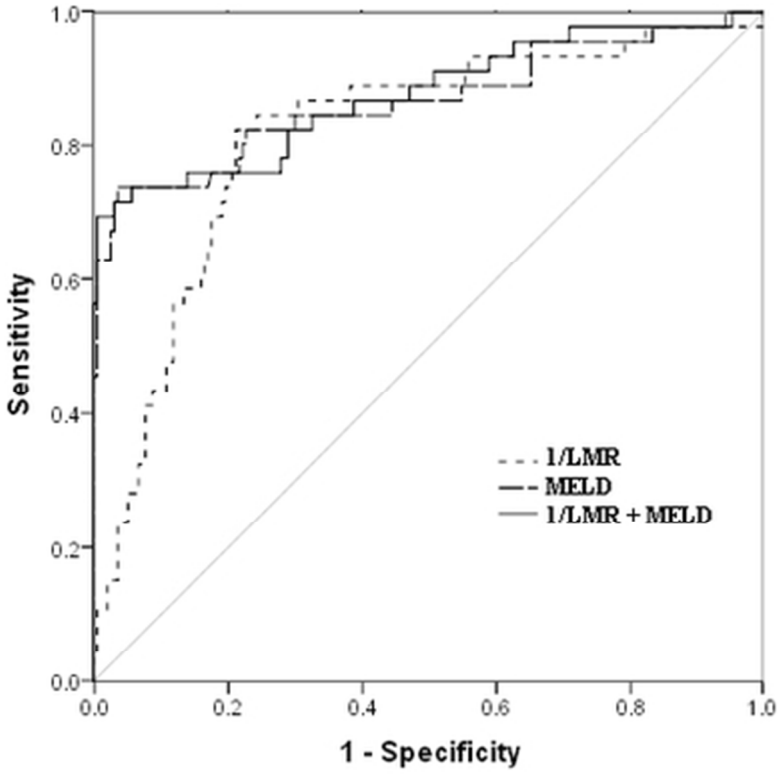

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STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	no
Study size	10	Explain how the study size was arrived at	no
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5-6
		(*b*) Describe any methods used to examine subgroups and interactions	5-6
		(*c*) Explain how missing data were addressed	no
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	5-6

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	5-6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5,table 1
		(b) Give reasons for non-participation at each stage	no
		(c) Consider use of a flow diagram	no
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5,table 1
		(b) Indicate number of participants with missing data for each variable of interest	no
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	no
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	5-6,table 1-2
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6,table 3
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-7,table 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
Discussion			
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8-9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-8
Generalisability	21	Discuss the generalisability (external validity) of the study results	7-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.

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Association between lymphocyte to monocyte ratio (LMR) and the mortality of HBV-related liver cirrhosis: A retrospective cohort study

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Abstract

Objective: Infection with hepatitis B virus (HBV) remains a major cause of liver cirrhosis (LC) in China. Recent reports suggest that the lymphocyte to monocyte ratio (LMR) is a potential biomarker for predicting clinical outcomes. In our study, we investigated if LMR can be used as a prognostic marker of mortality in HBV-related LC patients.

Design: A retrospective cohort study.

Setting: HBV-infected patients with LC and patients with chronic hepatitis B infection (CHB) from the Department of Infectious Disease were enrolled and 240 healthy individuals were recruited from the healthcare center at the First Affiliated Hospital of Zhejiang University.

Participants: 479 HBV-infected patients with LC, 134 patients with CHB, and 240 healthy individuals were enrolled.

Primary and secondary outcome measures: The receiver operating characteristic (ROC) curve and multivariable logistic regression analysis after adjusting total protein, albumin, total bilirubin and the model for end-stage liver disease (MELD) score, were used to evaluate the power of LMR for predicting 1-year mortality in LC patients.

Results: The LMR was statistically lower in LC patients. The MELD score and mortality were statistically higher in LC patients compared to those with chronic hepatitis B (CHB) and control groups. The area under the ROC curve (AUROC), cutoff values, sensitivity, and specificity of LMR for predicting mortality LC in the training cohort were 0.817 (95% confidence interval (CI): 0.746 - 0.888; $P < 0.001$), 2.10, 82.6, and 78.8%, and these data were confirmed in the validation cohort. The multivariate logistic regression analysis showed that LMR was an independent predictive factor of mortality in LC (odds ratios [OR]: 2.370, 95% CI: [1.070-5.249]; $P = 0.033$).

Conclusion: Our results strongly suggest that low LMR can be considered as an independent biomarker for predicting mortality in patients with LC.

Keywords: liver cirrhosis; lymphocyte to monocyte ratio; the model for end-stage liver disease score

Strengths and limitations of this study

- LMR was lower in the LC group, especially in the non-surviving group, compared to the control group and the CHB group.
- LMR was closely correlated to the MELD score.
- LMR was an easy parameter to achieve and the power for predicting mortality of LMR was similar to that of MELD.
- Low LMR levels were independent factors for predicting mortality in LC patients.
- This was a retrospective study.

Introduction

Liver cirrhosis (LC) is a common hepatic disease in China, and represents an increasing cause of morbidity and mortality [1,2]. Hepatitis B virus (HBV) infection remains a major cause of LC in China, with a 3% yearly incidence of decompensated cirrhosis [3]. Systemic inflammatory response syndrome (SIRS) is relatively common in patients with complicated cirrhosis [4,5]. SIRS can further deteriorate liver function, maximize the risk of complications and increase the mortality rate of LC patients [4,5]. SIRS is usually measured by peripheral blood count-based parameters, such as neutrophils, lymphocytes, monocytes, red blood cell distribution width (RDW), mean platelet volume (MPV) or platelet count. These parameters have been reported to be independent predictive markers of clinical outcome in cancer and different states of HBV-related hepatic disorders [6-10]. Among these inflammatory parameters, the neutrophil-lymphocyte ratio (NLR), RDW and monocyte ratio have been proposed as easily accessible and reliable markers [6-8,11]. Several recent studies suggest that the lymphocyte to monocyte ratio (LMR) is a cheap, readily available and reproducible test with potential for predicting clinical outcomes of patients with solid tumors and hematologic malignancy, including nasopharyngeal carcinoma, colorectal cancer, pancreatic cancer, and lymphoma [12-15]. Moreover, Merekoulias *et al.,* found that, in 90% of patients who had influenza virus, lymphopenia and/or monocytosis, LMR could be used as a time-saving and cost-effective screening test for influenza virus infection, leading to early antiviral treatment and a decreased incidence of complications [16]. Assuming that there may be association between LMR and LC severity, we investigated the potential prognostic value of LMR as a biomarker in HBV-related LC.

To the best of our knowledge, there is no data on LMR as a LC diagnostic measure. We therefore performed a retrospective cohort study to investigate, the association between LMR in peripheral blood in LC patients, with special emphasis on the value of LMR for predicting the mortality of LC patients.

Subjects and Methods

Subjects

We continuously analyzed all 547 patients with HBV-related liver cirrhosis (LC) from the Department of Infectious Disease, The First Affiliated Hospital, School of Medicine, Zhejiang University, between October 2012 and October 2013. Sixty-eight LC patients with a concurrent infection of hepatitis C/D/E/G virus (n = 3), human immunodeficiency virus (HIV, n = 1), hepatocellular carcinoma (HCC, n = 56), alcoholic cirrhosis (n = 5), schistosomiasis cirrhosis (n = 1), and any autoimmune liver disease (n = 2) were excluded. The remaining 479 LC patients were enrolled in our retrospective cohort study. All clinical data were retrieved from medical records at the Department of Infectious Disease. One hundred thirty-four patients with chronic hepatitis B infection (CHB), with no statistical differences in age and gender versus LC patients, were selected from the Department of Infectious Disease without a concurrent infection of hepatitis C/D/G virus, HIV, HCC, and any autoimmune liver

disease between October 2012 and October 2013. CHB and LC were diagnosed according to the criteria of the 2000 Xi'an viral hepatitis management scheme [17]. Liver cirrhosis was diagnosed based on the history of liver disease, clinical manifestations, laboratory tests, imaging tests, and, whenever feasible, liver biopsy[17]. Decompensated cirrhosis was defined by the presence of jaundice, ascites, variceal haemorrhage or hepatic encephalopathy[17-18]. The causes of admission in LC patients *without* decompensation were mainly jaundice, hypodynamia, and portal hypertension manifestations (esophageal varices, hypersplenism). The causes of admission in LC patients *with* decompensation were ascites, upper gastrointestinal bleeding (esophageal varices), hepatic encephalopathy, hepato-renal syndrome, and infection. CHB was defined as hepatitis B or hepatitis B surface antigen (HBsAg) positivity for > 6 months, and persistently positive HBsAg and/or HBV DNA [17-18]. The LC group was subdivided into two subgroups according to mortality at 1-year of follow up. 227 LC and 33 CHB were under antiviral therapy before admission, 189 LC patients and 76 CHB were under antiviral therapy after admission, altogether 416 LC (86.8%) and 109 (81.3%) CHB were under antiviral therapy. For LC and CHB patients discharged from hospital, 1-year prognostic information was obtained by checking medical records or by contacting the patients' family members. One hundred and eight LC patients were decompensated. Out of 92 LC patients died of upper gastrointestinal bleeding (n = 40), hepatic encephalopathy (n = 28), hepato-renal syndrome (n = 15), infection (n = 5), electrolyte disturbance (n=2), multiple organ failure (n=1), and respiratory failure (n=1). Two hundred and forty healthy controls with no statistical differences in age and gender versus LC patients were selected from health examination population who underwent a general health checkup that included a physical examination and some clinical laboratory tests at the Health Care Centre of the First Affiliated Hospital of Medical College of Zhejiang University between September 2013 and October 2013. They corresponded to HBsAg negative individuals with normal liver function, normal renal function, and no infection. One hundred and thirty-four CHB patients and 240 healthy controls were used to compare basic characteristics with 479 LC patients.

Ethics statement

This study was approved by the Ethics Committee of the First Affiliated Hospital of the Medical College at Zhejiang University in China and was performed in accordance with the Helsinki Declaration.

Laboratory assessment

All venous blood samples were obtained in the morning following a 12 h fast, within 24 h after admission. All study participants were subjected to the following determinations: serum total protein (TP), albumin (ALB), total bilirubin (TB), alanine aminotransferase (ALT), aspartate aminotransferase (AST), triglyceride (TG), total cholesterol (Tch), creatinine (Cr), prothrombin time (PT), complete blood cell counts, LMR in peripheral blood), international normalized ratio (INR) and the model for end-stage liver disease (MELD) score based on TB, Cr, INR and PT [18]. Complete

blood cell counts were determined using a Sysmex XE-2100 automated hematology analyzer (Sysmex Corp, Kobe, Japan) with Sysmex reagents.

Statistical analysis

Statistical analysis was performed using SPSS16.0 (SPSS Inc. IL, USA). Data were presented as mean ± SD, median (range) or categorical data as percentages, if appropriate. The differences between two groups were assessed with an independent sample t-test, the Mann-Whitney U test or chi-square test, if appropriate. Multiple comparisons were performed by one-way analysis of variance (ANOVA) or Kruskal-Wallis H tests, if appropriate. The LC cohorts were randomly divided into estimation and validation cohorts by random number generators. Spearman correlation test was used in correlation analyses. The receiver operating characteristic (ROC) curve and cutoff values of LMR were obtained, and area under ROC curve (AUROC) was calculated to identify the best LMR and/or MELD score for predicting mortality in LC patients. For AUROC analysis of combined 1/LMR and MELD score for predicting mortality in LC patients, predictive models of 1/LMR, MELD, and 1/LMR + MELD were first developed by binary logistic regression analyses, respectively. Probabilities of 1/LMR, MELD, and 1/LMR + MELD were then generated, respectively, and used as three new input variables for the ROC curve analysis (shown in Figure 2). These parameters were selected by stepwise regression, and multivariate logistic regression analyses were used to evaluate if low LMR was an independent factor for predicting mortality in LC patients by an unadjusted model and adjusting for TP, ALB, TB and MELD score. The high LMR group was used as the reference category. Statistical significance was defined at $P < 0.05$.

Results

Patient characteristics

There were 479 LC patients, 134 CHB patients, and 240 healthy controls enrolled in our study. The patient characteristics are listed in Table 1. No statistical differences were observed for gender and age between the three groups. Whereas, TP, ALB, TB, ALT, AST, TG, Tch, Cr, INR, LMR, and WBC count had statistically differences (all $P < 0.05$). The MELD score and mortality of the LC group were statistically higher than those of the CHB group ($P < 0.001$).

LMR is lower in LC, especially in the non-surviving group

The LMR was significantly lower in the LC group compared to the control group (2.77 *vs.* 5.30, respectively) and to the CHB group (2.77 *vs.* 3.64; $P < 0.01$). The clinical characteristics and differences in variables between non-surviving and surviving LC patients are presented in Table 2. The non-surviving patients had lower LMR (Fig. 1), TP, ALB, and Tch, and higher MELD score, TB, ALT, AST, TG, Cr, INR, WBC, monocytes, and rate of decompensated cirrhosis, compared with surviving patients. The median and range of lymphocyte count of the non-surviving group were slightly lower than those of the surviving group, but the difference did not

reach statistical significance. These data indicate that the lower LMR in the non-surviving group was mainly due to an increased number of monocytes and secondarily due to decreased lymphocytes. LMR resulted in no significant differences in LC patients whose primary cause of death was upper gastrointestinal bleeding, hepatic encephalopathy, or hepato-renal syndrome ((1.35[0.35-17.75]), 1.42[0.27-18.20], 1.39[0.39-18.25], p=0.955).

LMR is correlated to the MELD score

The LMR in the LC group negatively correlated with MELD score (r = -0.241; $P < 0.05$), especially in non-surviving LC patients, LMR negatively correlated with MELD score with a higher correlation coefficient (r = -0.354; $P = 0.013$) compared with LMR in surviving LC patients.

The power for predicting 1-year mortality of LMR

The enrolled 479 LC patients were randomly divided into two cohorts: the training cohort (n = 239) and the validation cohort (n = 240). The ROC curve analyses of the training cohort were applied to estimate LMR and MELD score to predict mortality of LC patients (Fig. 2). LMR was changed into 1/LMR by inverse transformation. The AUROCs of 1/LMR and MELD score were 0.817 (95% confidence interval (CI): 0.746 - 0.888; $P < 0.001$) and 0.868 (95% CI: 0.795 - 0.941; $P < 0.001$), respectively. The cutoff values, sensitivity and specificity of MELD were 19.1, 73.9 and 96.4%. LMR values were 2.10, 82.6, and 78.8%. When 1/LMR and MELD score were combined, the AUC was 0.876 (95% CI: 0.808 - 0.945; $P < 0.001$), only slightly higher than AUC of MELD score, and neither the specificity (71.7%) nor the sensitivity (96.9%) was significantly improved. Applying the LMR to the validation cohort, the AUROCs of 1/LMR, MELD score, and 1/LMR+MELD were 0.773 (95% CI: 0. 692 - 0.854; $P < 0.001$), 0.887 (95% CI: 0.829 - 0.945; $P < 0.001$), 0.890 (95% CI: 0.836 - 0.944; $P < 0.001$), respectively. There were no significant differences in the AUCs of LMR between the estimation and validation cohorts ($Z = 0.741$, $P = 0.053$). To summarize, LMR was an easy parameter to achieve and the power for predicting mortality of LMR was similar to that of MELD.

LMR is an independent prognostic factor of mortality in multivariate analysis

MELD, low LMR (LMR ≤ 2.10, with high LMR > 2.10 as a reference), TP, TB and ALB were selected by stepwise regression from the above parameters ($P = 0.025$, < 0.001, 0.048, 0.006, < 0.001 and 0.021, respectively) with forward selection. Subsequent multivariate logistic regression analysis showed that low LMR was an independent factor for predicting mortality in LC patients (Table 3).

Discussion

In the present retrospective study of HBV-LC a significant negative association was found between LMR in the peripheral blood and the MELD score. LMR of LC patients was statistically lower, and the MELD score and mortality of LC patients were statistically higher than those of CHB and control groups, especially in the

non-surviving LC subgroup. Moreover, low LMR was an independent predictive factor of mortality. These results provide the first evidence for an association between LMR and mortality in LC patients.

Each year approximately 2%-5% of compensated cirrhosis patients develop decompensation, decompensated cirrhosis patients mainly die of cirrhosis-related complications, and the prognosis of decompensated cirrhosis is markedly worse, with a 5-year survival of 14%-35% compared to 84% in compensated cirrhosis[19-20]. Decompensated cirrhosis patients frequently present with more than one facet of liver decompensation, and should then receive liver intense medical care and transplantation evaluation[19]. In our non-surviving group, most patients had decompensated cirrhosis, and their LMR values were significantly lower than those of the surviving group where most patients had compensated cirrhosis. LMR was significant correlated to the MELD score with a low (r) correlation coefficient. However the r value in non-surviving LC patients was higher than in surviving LC patients, indicating that the LMR changes in non-surviving LC patients were more pronounced, which coincided with Table 3 results.

Bacterial infections are an important cause of morbidity and mortality in patients with LC due to an impaired immune function together with an increased passage of bacteria from the gut (bacterial translocation [BT]) [4,5,21]. Once infection occurs, it may lead to SIRS, which can cause serious complications such as severe sepsis, renal dysfunction, encephalopathy, coagulopathy and multiple organ failure [21]. SIRS occurs more frequently in patients with advanced cirrhosis and portal hypertension, and is associated with severity of liver disease and increased risk of death in LC patients [4,5]. The mortality of LC patients with infection has been reported to be more than twice that of patients without infection [21]. Monocytes are central mediators of the immune response and play a crucial role in the pathogenesis of liver cirrhosis. Endotoxin leads to monocyte activation and promotes the release of proinflammatory cytokines such as interleukin-1 (IL-1), IL-6, tumor necrosis factor-α (TNFα), and interferon-γ into the serum. This release is proportional to liver disease severity. These cytokines act in an autocrine and paracrine fashion and result in the recruitment of inflammatory effector cells, such as polymorphonuclear cells [21-23]. The subsequent activation of nitric oxide (NO) *via* the cytokine cascade leads to vasodilatation [24]. Endotoxin, cytokines and NO are key elements in the pathogenesis of circulatory abnormalities in liver cirrhosis with infection. Li *et al.,* found that monocytes in HBV-related LC patients positively correlated with the endotoxin level and cirrhosis severity based on the Child-pugh classification, indicating that the endotoxin-driven monocyte activation was an important factor of SIRS and multiple organ failure [25]. Lee *et al.*, found that LC patients with hepatocellular carcinoma had a high monocyte ratio and that a preoperative monocyte ratio > 7% was an independent risk factor for survival after hepatic resection [11]. Immune paralysis, defined as decreased human leukocyte antigen-DR (HLA-DR) expression on monocytes and indicating immune dysfunction, was found in LC patients. HLA-DR expression is a direct marker of monocyte function and a protective immune response in LC patients [26]. Monocyte HLA-DR expression is significantly reduced in those patients and falls in proportion to cirrhosis

severity [27,28]. Therefore, LC patients may have high monocyte count but low monocyte HLA-DR expression for systemic inflammatory response and immune paralysis. Early diagnosis and treatment of infections can significantly reduce morbidity and improve survival of LC patients [4,5,22,24,25,29].

Inflammatory stimuli mainly affect the numbers of monocytes in the peripheral blood in LC patients, which contributes to LMR changes. In addition, the present study showed that lymphocytes in the non-survival group showed a trend towards lower levels as compared with the survival group, without reaching statistical significance. Such a decline might be attributed to lymphocytopenia [30,31]. This is in accordance with Leithead *et al.,* who found that a lower lymphocyte count was associated with mortality in patients with end-stage cirrhosis listed for liver transplantation [28]. Lombardo *et al.,* also found that the progressive and severity-related decrease in peripheral blood T-lymphocyte suggested a progressive impairment of protective immune function in LC [31]. Therefore, high monocytes together with low lymphocytes may reflect the severity and progression of liver injury in LC patients.

LMR has been shown to be associated with tuberculosis and influenza virus infection [16,32]. Recently, LMR has also been reported to predict survival and prognosis in various patient populations with malignant diseases [12-15], and a decreased LMR has been shown to be significantly associated with a high risk for critical limb ischemia in peripheral arterial occlusive disease patients [33]. Compared with another novel inflammation index, the ability of NLR for predicting mortality (AUROC) in LC patients [34] was similar to LMR in our study. LMR was associated, in our study, with MELD score, the power for predicting mortality of LMR was similar to that of MELD, and was an independent predictive factor of mortality. In addition, the LMR is an easily available and low price biomarker. However, it should be noted that this was a retrospective study so that prospective cohorts are warranted in order to confirm the present data. Another study limitation was a 1:1 ratio was not adopted for setting up the control groups. Moreover, these findings may only apply to HBV-related LC patients and, therefore, need to be validated in other etiologies of liver cirrhosis by future prospective clinical trials.

Contributorship statement L.F. designed the experiments. G. F., JW.Z. J.Z performed the experiments. Y.Z. and J.Y. analyzed and interpreted all the data. J.Z and Y.Z. wrote the main manuscript text. All authors reviewed the manuscript.

Competing interests None declared.

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Ethics approval This study was approved by the ethics committee of the First Affiliated Hospital of Zhejiang University School of Medicine, China. Patient consent Obtained.

Data sharing statement No additional unpublished data are available.

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Figure Legends

Figure 1: The boxplots of MELD score and LMR between surviving and non-surviving LC patients
LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score.

Figure 2: Receiver operating characteristic (ROC) curve analysis for predicting mortality by LMR and MELD score in the training cohort.
LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score; 1/LMR+MELD, 1/LMR combined with MELD.

Table 1. Basic characteristics of enrolled participants.

Variables	Control (240)	CHB (134)	LC (479)	*P* value
Female/male	61/179	34/100	126/353	0.956
Age (year)	50.6±9.69	48.9±8.04	50.8±10.8	0.163
HBsAg positive (yes/no)	0/240	134/0	479/0	-
HBeAg positive (yes/no)	0/240	66/68	184/295	0.024#
TP (g/L)	71.6±3.79	67.3±6.83*	62.9±8.48*#	<0.001
ALB (g/L)	46.2±3.17	37.4±5.95*	33.2±5.61*#	<0.001
TBIL (μmol/L)	12(6-49)	21.5(5-309)*	31(5-839)*#	<0.001
ALT (U/L)	17(7-48)	61(9-1838)*	29(4-1882)*#	<0.001
AST (U/L)	19(12-46)	48(16-1235)*	40(8-4094)*#	<0.001
TG (mmol/L)	1.08(0.41-1.70)	1.33(0.44-4.14)*	0.79(0.3-3.59)*#	<0.001
Tch (mmol/L)	4.66(2.40-5.86)	4.04(1.6-8.17)*	2.89(0.74-9.73)*	<0.001
Cr (μmol/L)	73(39-100)	65(29-154)*	66(30-729)*	0.002
INR	0.94±0.05	1.21±0.23*	1.55±0.78*#	<0.001
WBC (10^{12}/L)	5.6(4.0-9.4)	4.75(2-12)*	3.9(0.8-32.8)*#	<0.001
LMR	5.30(1.4-13.2)	3.64(0.65-9.61)*	2.77(0.27-18.25)*#	<0.001
MELD score	-	5.89(0-23.63)	9.89(0-57.17)	<0.001 #
Mortality (yes/no)	-	1/133	92/387	<0.001 #

Data were presented as mean ± SD and median (range). CHB, chronic hepatitis B; LC, liver cirrhosis; HBsAg, hepatitis B surface antigen; HBeAg, hepatitis B e antigen; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; LMR, lymphocyte to monocyte ratio; MELD score, model for end-stage liver disease score. *P*-value: Comparison among these three groups. #: LC group *vs.* CHB group. *: $P < 0.05$ *vs.* the Control group

Table 2. The clinical characteristics and differences in variables between non-surviving and surviving LC patients.

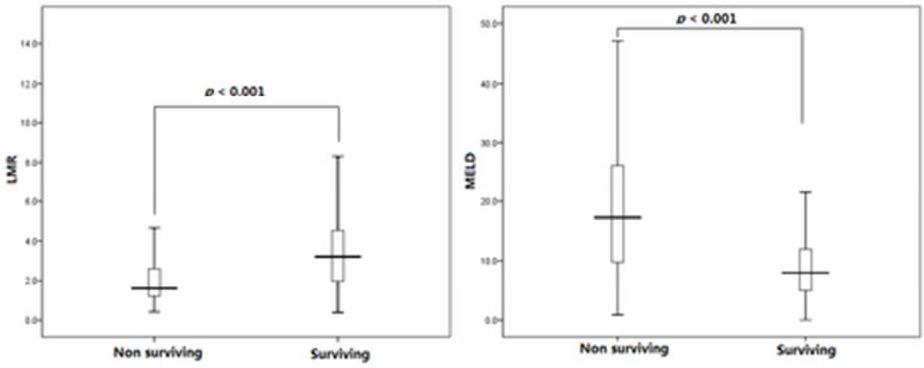
Variables	Non-surviving (n=92)	Surviving (n=387)	*P* value
Female/male	30/62	96/291	0.127
Age (year)	53.8±10.3	50.1±10.8	0.003
TP (g/L)	56.4±8.40	64.5±7.74	<0.001
ALB (g/L)	29.7±5.17	34.0±5.40	<0.001
TBIL (μmol/L)	292.5(9-839)	27(5-836)	<0.001
ALT (U/L)	48(4-1882)	27(5-475)	<0.001
AST (U/L)	66(10-4094)	37(8-440)	<0.001
TG (mmol/L)	0.88(0.30-2.15)	0.76(0.33-3.59)	0.022
Tch (mmol/L)	1.83(0.74-5.29)	3.02(0.94-9.73)	<0.001
Cr (μmol/L)	73.5(30-729)	65(30-326)	<0.001
INR	2.23±1.51	1.39±0.28	<0.001
WBC (10^9/L)	6.75(0.8-24.9)	3.6(0.9-32.8)	<0.001
Monocytes(10^9/L)	0.73 (0.04-3.16)	0.33 (0.05-2.0)	<0.001
Lymphocyte(10^9/L)	0.9 (0.1-4.3)	1.00 (0.10-5.40)	0.166
LMR	1.41(0.27-18.25)	3.10(0.38-14.58)	<0.001
MELD score	22.94(0.84-57.17)	8.49(0-35.33)	<0.001
Decompensated cirrhosis (yes/no)	82/10	26/361	<0.001

Data were presented as mean ± SD and median (range). LMR, lymphocyte to monocyte ratio; LC, liver cirrhosis; TP, total protein; ALB, Albumin; TB, total bilirubin; ALT, alanine aminotransferase; AST, aspartate aminotransferase; TG, triglyceride; Tch, total cholesterol; Cr, creatinine; INR, international normalized ratio; WBC, white blood cell; MELD score, model for end-stage liver disease score.

Table 3. Odds ratios of low LMR for predicting mortality in LC patients

Models	Odds Ratio (95% CI)	*P* value
Model 1	8.623 (5.051-14.721)	< 0.001
Model 2	3.324 (1.571-7.035)	< 0.001
Model 3	2.370 (1.070-5.249)	0.033

Odds ratios of low LMR were determined using high LMR as reference; model 1: unadjusted; model 2: adjusted for TP, ALB, and TB; model 3: adjusted for TP, ALB, TB and MELD score.



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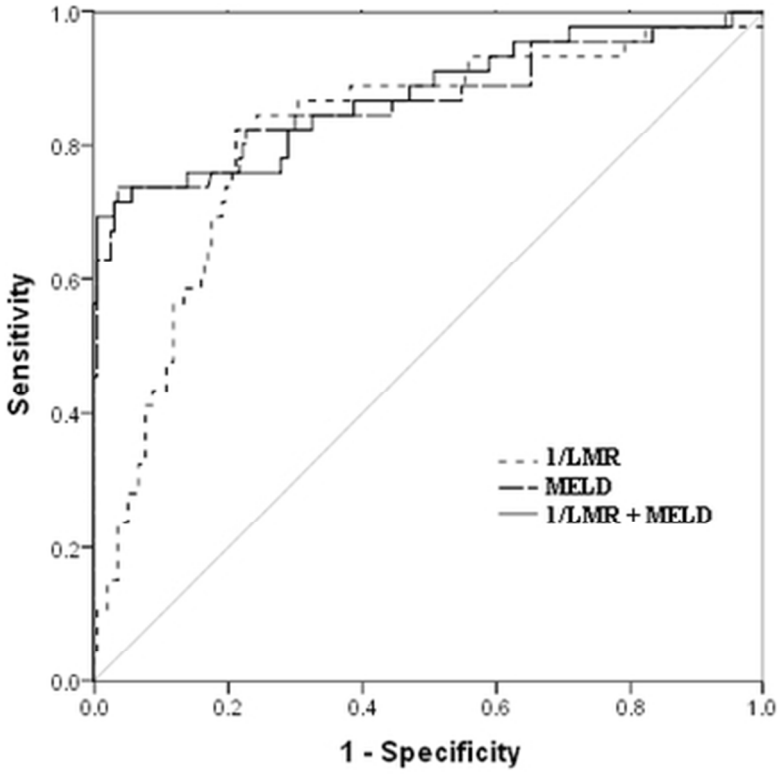

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STROBE 2007 (v4) checklist of items to be included in reports of observational studies in epidemiology*

Checklist for cohort, case-control, and cross-sectional studies (combined)

Section/Topic	Item #	Recommendation	Reported on page #
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1-2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	1-2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	4
Objectives	3	State specific objectives, including any pre-specified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	4-5
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	4-5
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-5
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	*5*
Bias	9	Describe any efforts to address potential sources of bias	no
Study size	10	Explain how the study size was arrived at	no
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	5
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	5-6
		(*b*) Describe any methods used to examine subgroups and interactions	5-6
		(*c*) Explain how missing data were addressed	no
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed	5-6

		Cross-sectional study—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	5-6
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	5,table 1
		(b) Give reasons for non-participation at each stage	no
		(c) Consider use of a flow diagram	no
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	5,table 1
		(b) Indicate number of participants with missing data for each variable of interest	no
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	no
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	5-6,table 1-2
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	6,table 3
		(*b*) Report category boundaries when continuous variables were categorized	6
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	6-7,table 3
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	6
Discussion			
Key results	18	Summarise key results with reference to study objectives	7
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	8-9
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	7-8
Generalisability	21	Discuss the generalisability (external validity) of the study results	7-9
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	9

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.
Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.